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Q1534

**From:** Davis, Minh-Tam  
**Sent:** Friday, October 11, 2002 12:58 PM  
**To:** STIC-ILL  
**Subject:** FW: reprint request for 09/824134

Please add another reference.

Kasahara, N, 1994, Science, 266 (5189): 1373-6.

Thank you.

MINH TAM DAVIS  
ART UNIT 1642, ROOM 8A01, MB 8E12  
305-2008

-----Original Message-----

**From:** Davis, Minh-Tam  
**Sent:** Friday, October 11, 2002 12:41 PM  
**To:** STIC-ILL  
**Subject:** reprint request for 09/824134

Michael, SI, 1994, Gene therapy, 1(4): 223-32

Thank YOU.

MINH TAM DAVIS  
ART UNIT 1642, ROOM 8A01, MB 8E12  
305-2008

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9. W1118 flies that lacked screening pigment were raised at 25°C either under constant white (room light) illumination or in darkness. Adult flies were plunged into a liquid nitrogen bath with vigorous shaking after either a 20-min incubation in the dark or 20 min of blue illumination. All further manipulations were done either in the dark or in dim red light. The heads were then separated from the bodies by passing the mixture through steel sieves with a mesh size of 710 µm. Collected heads were disrupted on ice in a Teflon-glass homogenizer with buffer (pH 6.7) containing 250 mM sucrose, 120 mM KCl, 10 mM MOPS, 5 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, leupeptin (10 µg/ml), pepstatin (1.2 µg/ml), and 1 mM phenylmethylsulfonyl fluoride. The resulting suspension was centrifuged twice at 10000g for 5 min at 4°C to remove unhomogenized particulate material. Membranes were collected from the supernatant by centrifugation at 45,000g for 1 hour and resuspended in homogenization buffer. All difference spectra shown in this figure were recorded at 25°C in a UV-2101 double-beam Shimadzu spectrophotometer with an unilluminated sample as the reference. Approximately 1400 fly heads were used for each experiment.

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11. A 150-watt fiber optic illuminator (Cole-Parmer, Chicago, IL) equipped with cut-off filters (Oriel, Stamford, CT) was used as the light source for all experiments. For blue illumination, a combination of blue and yellow filters (BG-12 and GG420) was used to transmit wavelengths from 400 to 450 nm. For red illumination, a red filter (RG610) was used to cut off wavelengths below 610 nm. For dim red illumination and other darkroom operations, a Kodak safety lamp with a GBX-2 red filter was used.

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16. At present, we cannot exclude the possibility that the intermediate that activates G proteins is not metarhodopsin but one that is formed at some stage during the decay of metarhodopsin into retinal and opsin. For example, G protein activation could be triggered by a short-lived species with a deprotonated Schiff base in analogy with the activation pathway in the vertebrate visual cycle.

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19. Incubation of membranes from illuminated flies with urea (2.5 M, 25°C) resulted in the almost complete decay of metarhodopsin within 20 min. During this decay, arrestin was released from the membranes into the aqueous phase, consistent with its proposed role in stabilizing metarhodopsin.

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29. Supported by grants from the National Eye Institute and the Seale Scholars Program-The Chicago Community Trust. We thank Y. Long for the assistance with maintenance of fly stocks and J. Delaney, R. Jager, R. Henderson, J. Heymann, J. Milne, C. Montell, J. Nathans, and K.-W. Yau for helpful comments on the manuscript.

8 July 1994; accepted 26 September 1994

## Tissue-Specific Targeting of Retroviral Vectors Through Ligand-Receptor Interactions

Noriyuki Kasahara, Andrée M. Dozy, Yuet Wai Kan\*

The development of retroviral vectors that target specific cell types could have important implications for the design of gene therapy strategies. A chimeric protein containing the polypeptide hormone erythropoietin and part of the env protein of ecotropic Moloney murine leukemia virus was engineered into the virus. This murine virus became several times more infectious for murine cells bearing the erythropoietin receptor, and it also became infectious for human cells bearing the erythropoietin receptor. This type of tissue-specific targeting by means of ligand-receptor interactions may have broad applications to a variety of gene delivery systems.

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**M**ammalian retrovirus vectors commonly used for gene transfer are classified on the basis of their host range as either ecotropic, which only infect murine cells, or amphotropic, which infect both murine and nonmurine cells. The host range is determined primarily by the binding interaction between viral envelope glycoproteins and specific proteins on the host cell surface that act as viral receptors (1). In murine cells, an amino acid transporter serves as the receptor for the envelope glycoprotein gp70 of ecotropic Moloney murine leukemia virus (Mo-MuLV) (2). The receptor for the amphotropic Mo-MuLV has recently been cloned and shows homology to a phosphate transporter (3). Because the transporters are widely distributed among various tissues, these retrovirus vectors can infect virtually all cell types and therefore are not tissue-specific. It has previously been shown that the host range of viruses can be altered by pseudotyping (4). However, the alternative envelope proteins used in such experiments were derived from naturally occurring viral sequences such as those of gibbon ape leukemia virus, avian leukosis virus, and the human immunodeficiency virus, and hence the resultant pseudotyped virions were still limited by the host range of the naturally occurring virus. In some cases, it has been shown that viral targeting can be achieved by ligand-receptor interactions, mediated by bivalent antibodies linked by biotin-streptavidin (5) or by chemical modification with lactose to produce an asialoglycoprotein (6). These manipulations, which involve modifications to the virus after its production, usually result in low infection efficiency. A recombinant virus containing in its envelope a sequence encoding a single-chain antibody variable region has been shown to bind to a solid matrix containing the appropriate polypeptide antigen, and the bound viruses, as expected, were infectious for NIH 3T3 cells (7). However, direct infection of target cells by the virus through antigen-antibody interaction was not demonstrated. In this study, we engineered an ecotropic virus to bear a chimeric envelope-ligand protein on its surface. This virus not only showed enhanced infectivity for murine cells that bear the appropriate receptor but could also cross species and specifically infect the appropriate receptor-bearing human cells.

We introduced the polypeptide hormone erythropoietin (EPO) into the ecotropic Mo-MuLV envelope. A portion of the Mo-MuLV envelope gene (*env*) (8) encoding the NH<sub>2</sub>-terminal end of gp70 was removed and replaced, in frame, with sequences coding for EPO (9); this construct was designated pEPOenv Δ5923. Portions of the gp70 sequence that encode the *env* signal peptide at the NH<sub>2</sub>-terminus, as well as the cysteine residues in the COOH-terminal region that participate in sulphydryl bonding with the inner envelope subunit p15, were left intact. This EPO-*env* hybrid construct was cotransfected into ψ2 packaging cells (10) with the

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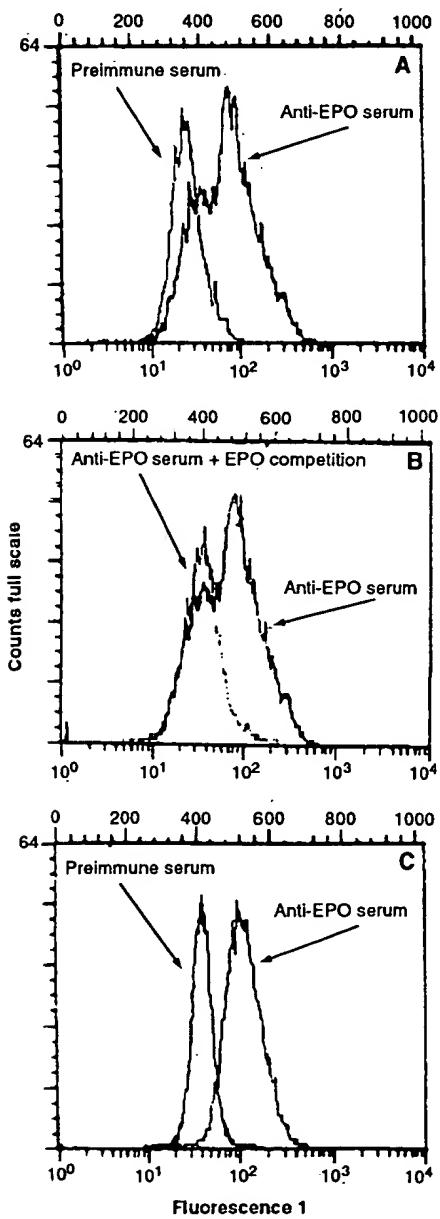
otrexate (MTX)-resistant dihydrofolate reductase gene (*dhfr*) (11). The transfected cells were selected with increasing concentrations of MTX, and subclones were isolated and screened by protein immunoblot analysis of whole-cell lysates; an EPO-specific monoclonal antibody (mAb) and a polyclonal *env*-specific antiserum (anti-*env*) were used. Several of the subclones transfected with pEPOenv Δ5923 showed the same 70-kD band, which reacted both with the EPO mAb and the anti-*env*, which indicates coexpression of both epitopes in the same protein (12). One subclone, designated ψEPOenv 8, was chosen for further characterization.

To demonstrate that the chimeric envelope protein was transported to and oriented correctly in the cell membrane, we analyzed ψEPOenv 8 cells by fluorescence-activated

cell sorter (FACS) analysis. A biphasic pattern of fluorescence was observed with antisera to EPO (anti-EPO). One peak was shifted (Fig. 1A), and the shift was abolished in competition experiments with an excess of EPO peptide (Fig. 1B). We sorted the cells showing high fluorescence intensity to obtain a monophasic population of cells expressing the EPO epitope at high concentrations (Fig. 1C). The monophasic shift in fluorescence could also be abolished by competition with soluble EPO peptide (12). One subcloned packaging cell line showing the largest shift in fluorescence, designated ψEPOenv 8.1, was selected for further studies. Southern (DNA) blot analysis showed that this cell line, as expected, carried the 2.4-kb Eco RI restriction fragment containing the chimeric EPO-*env* sequence (12).

Virus-producing cell lines were then generated by infection of these packaging cells with virion-containing cell culture medium harvested after transient transfection of wild-type PA317 packaging cells (13) with pCRIP-SVlac, a packageable, replication-defective viral vector (14) that contains both the gene for neomycin resistance and the gene for β-galactosidase (β-Gal). G418-resistant subclones of ψEPOenv 8.1 were retested for expression of the EPO-*env* proteins by protein immunoblot and FACS analysis, and a positive cell line designated ψEPOenv 8.1.8 was obtained. Viruses produced by this cell line should be coated with wild-type ecotropic envelope proteins, derived from the parental ψ2 cell line, as well as the chimeric EPO-*env* proteins, derived from the stably transfected construct.

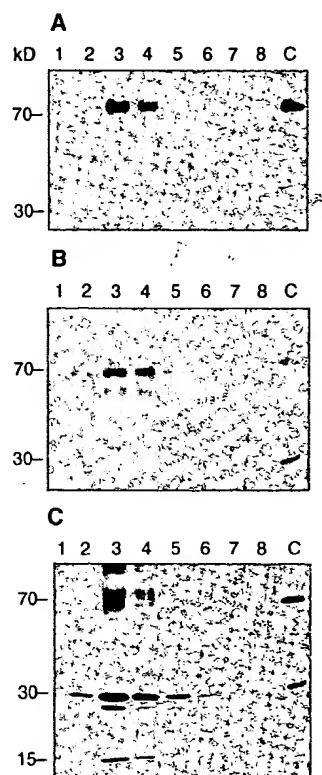
To show that the virions produced by these producer cell lines do contain the chimeric envelope protein, culture medium from these cell lines was fractionated by



**Fig. 1.** FACS analysis of packaging cell lines. Subclones of ψ2 packaging cells that had been stably transfected with the chimeric envelope construct pEPOenv Δ5923 (18) were initially screened for expression of the protein by protein immunoblot (19). Positive subclones were grown to confluence on 10-cm plates, detached and dispersed with PBS + 2 mM EDTA, and preincubated with normal goat serum in PBS + 2% BSA for 30 to 60 min on ice. After washing with PBS + 2% BSA, the cells were incubated with preimmune rabbit serum (CalTag) or with a polyclonal EPO-specific rabbit antiserum (no. 8C295, Amgen) in PBS + 2% BSA for 30 to 60 min, washed again, incubated with fluorescein-conjugated goat antiserum to rabbit IgG (CalTag), washed, and subjected to flow cytometry after addition of propidium iodide (20). (A) Before sorting, the ψEPOenv 8 cell line reacted with preimmune and EPO-specific antiserum. (B) ψEPOenv 8 cells reacted with EPO-specific antiserum, with and without competition by excess soluble EPO. (C) After sorting, the ψEPOenv 8.1 cell line, a subclone isolated after sorting of the high-intensity fluorescence population, reacted with preimmune and EPO-specific antiserum.

sucrose density gradient centrifugation. The fractions were subjected to protein immunoblot analysis with antisera specific for EPO, *env*, or total Mo-MuLV. The same 70-kD band was detected by anti-EPO as well as anti-*env*, predominantly in two fractions that also contained other components of the Mo-MuLV virus, such as the p15 transmembrane subunit of the envelope and the 30-kD *gag* proteins (Fig. 2, A through C). This demonstrates that the chimeric EPO-*env* proteins were indeed incorporated into intact virions released by the producer cells into the medium.

To test whether the EPO-*env*-containing viruses had increased affinity for cells with



**Fig. 2.** Sucrose density gradient fractionation of virus particles isolated from overnight cell culture medium of producer cell line ψEPOenv 8.1.8. Viral particles from producer cell line ψEPOenv 8.1.8 (21) were purified by centrifugation at 150,000*g* through 2 ml of 20% sucrose onto a 1-ml 60% sucrose cushion in an SW40 rotor for 1 hour at 4°C. Virus particles removed at the interface were diluted to 3 ml, layered on top of a 9-ml 20 to 45% sucrose gradient, and centrifuged at 150,000*g* overnight in an SW40 rotor at 4°C. Eight 1-ml fractions (lanes 1 to 8) collected from the bottom of this gradient were each diluted 1:3 before pelleting at 190,000*g* in an SW50 rotor for 1 hour at 4°C. Pelleted samples were lysed in lysis buffer, boiled, and analyzed by protein immunoblot (19) with the use of either (A) EPO-specific antibody, (B) *env*-specific antibody, or (C) total MLV-specific antibody. The control lane (lane C) contains a sample of whole-cell lysate from cell line ψEPOenv 8.1.8.

the EPO receptor, we created an EPO receptor-containing target cell line by stably transfecting wild-type NIH 3T3 cells with a complementary DNA (cDNA) encoding the EPO receptor (15). Subclones were screened by  $^{125}\text{I}$ -labeled EPO radioligand binding assay, and the subclones showing the largest amounts of  $^{125}\text{I}$ -EPO binding, corresponding to approximately 10,000 receptors per cell, were chosen for use in infection experiments. The wild-type and EPO receptor-containing NIH 3T3 target cells were infected with the CRIP-SVlac vector, packaged by wild-type  $\psi 2$  cells or produced by the  $\psi\text{EPOenv}$  8.1.8 producer cell line. The EPO-env virus showed a 6.1-fold increase in efficiency on NIH 3T3 + EPO receptor target cells as compared with  $\psi 2$  wild-type virus. This increased infection efficiency was mediated through the EPO ligand-receptor interaction, because the increase in infection events was abolished in a dose-dependent manner by addition of soluble EPO peptide at the time of infection (Fig. 3A).

Wild-type ecotropic viruses normally do

not infect human cells. To test whether the ecotropic virus pseudotyped by the chimeric envelope would infect human cells bearing the EPO receptor, we next tested  $\psi 2$ , PA317-, and  $\psi\text{EPOenv}$  8.1.8-packaged CRIP-SVlac virus for its ability to infect human cells with or without the EPO receptor. As expected,  $\psi 2$ -packaged virus, which infects NIH 3T3 cells in the presence or absence of the EPO receptor, did not infect HEL (human erythroleukemia) or HeLa (human cervical carcinoma) cells, whereas PA317-packaged virus infected all of the cell lines. The  $\psi\text{EPOenv}$  8.1.8-packaged ecotropic virus specifically infected the erythroid cell line HEL, which bears the EPO receptor, but did not infect HeLa cells, which do not bear the EPO receptor (Fig. 3B). We also found that this virus infected the human erythroid cell line K562, but not the human lymphocytic cell line Raji (12). The HEL and K562 cells contained approximately 1000 and 800 receptors per cell, respectively, as measured by  $^{125}\text{I}$ -EPO binding. Because the ecotropic virus bearing the

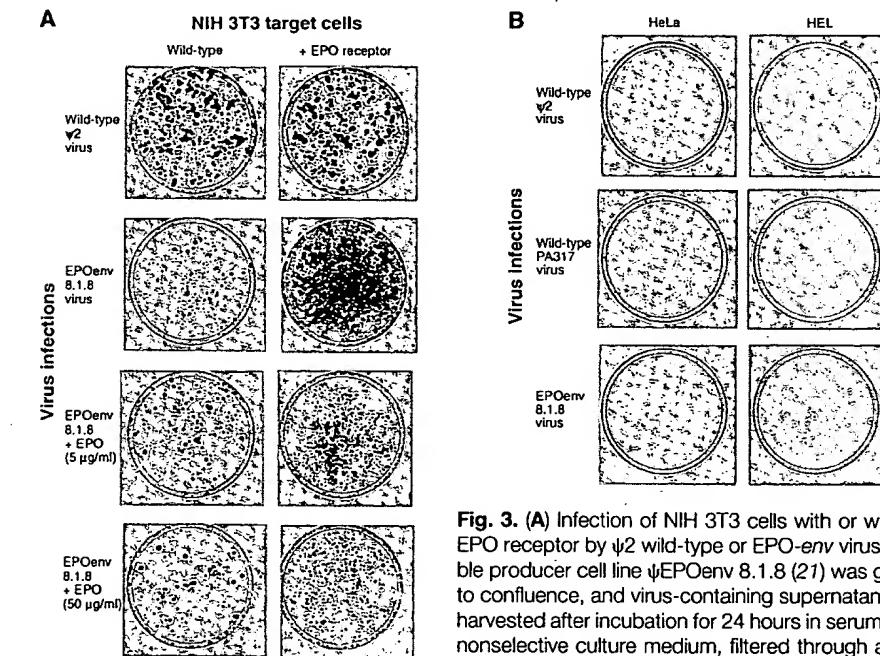
chimeric EPO-env envelope infected HEL and K562 cells as effectively as did the PA317-packaged amphotropic virus, the ligand-receptor interaction appears to have been highly efficient and did not require large numbers of receptors on the target cell surface.

Our present studies thus directly demonstrate enhanced ecotropic viral infection of murine cells expressing the appropriate receptor for EPO and, more strikingly, ligand-receptor-mediated, cell-specific, cross-species infection of EPO receptor-bearing human erythroid cells. Infection of earlier hematopoietic progenitors could conceivably be achieved by targeting c-Kit or CD34 (16). A retrovirus vector with tissue tropism for this specific subpopulation of target cells could potentially be of use in gene therapy for red blood cell disorders such as sickle cell anemia and thalassemias, as well as in developmental studies of erythroid cell differentiation. In principle, the approach demonstrated here should be broadly applicable, allowing production of a wide range of viruses that bear ligands for specific receptors and thus can deliver genes to specific tissues or organs for the treatment of genetic diseases and cancer.

*Note added in proof:* Since this manuscript was first submitted, Valsesia-Wittmann et al. (17) have reported infection of mammalian cells by avian retroviruses through introduction of an integrin sequence into the envelope.

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**Fig. 3.** (A) Infection of NIH 3T3 cells with or without EPO receptor by  $\psi 2$  wild-type or EPO-env virus. Stable producer cell line  $\psi\text{EPOenv}$  8.1.8 (21) was grown to confluence, and virus-containing supernatant was harvested after incubation for 24 hours in serum-free, nonselective culture medium, filtered through a 0.2- $\mu\text{m}$  syringe filter, and diluted 1:10 before being added

to  $5 \times 10^5$  NIH 3T3 wild-type or NIH 3T3 + EPO receptor target cells (22) in serum-free medium containing polybrene (8  $\mu\text{g}/\text{ml}$ ). Wild-type  $\psi 2$  cells stably expressing CRIP-SVlac virus after infection by means of PA317-derived vectors were used for production of  $\psi 2$  wild-type control virus. For competition studies, 5 to 50  $\mu\text{g}$  of soluble EPO (Amgen) was also added to the target cell plates at the time of infection. The infected target cells were trypsinized 24 hours later, replated at 1:50 dilution in duplicate, and selected with G418 (400  $\mu\text{g}/\text{ml}$ ) for 12 days, after which the plates were fixed with methanol and acetone (1:1), and the colonies were visualized by Giemsa staining and counted (23). (B) Infection of human cell lines with or without EPO receptor. Virus-containing supernatant was harvested from producer cell line  $\psi\text{EPOenv}$  8.1.8 (20) at confluence after overnight incubation in serum-free nonselective medium, filtered through a 0.2- $\mu\text{m}$  syringe filter, and added without dilution to  $5 \times 10^5$  HEL cells, which express EPO receptor (21), or HeLa cells, which do not express EPO receptor, in medium containing polybrene (8  $\mu\text{g}/\text{ml}$ ). Negative control infections were done with virus similarly harvested from wild-type  $\psi 2$  producer cells, and positive control infections were done with wild-type PA317 cells expressing CRIP-SVlac virus at a titer of  $6.8 \times 10^2$  per milliliter, diluted 1:10. The infected target cells were replated 24 hours later at a 1:5 dilution and selected with G418 (400 to 1000  $\mu\text{g}/\text{ml}$ ), after which the plates were fixed, and the surviving cells were visualized by Giemsa staining.

17. S. Valsesia-Wittmann et al., *J. Virol.* **68**, 4609 (1994).
18. Plasmid EPOenv A5923 was constructed by use of unique restriction sites at position 5923 (Bst EII) and position 6537 (Bam HI) of the Mo-MuLV *env* gene to delete the intervening envelope sequence and allow the EPO sequence to be inserted, in frame, at the NH<sub>2</sub>-terminal end of gp70. The EPO cDNA sequence coding for the mature 166-amino acid peptide hormone, without the 27-amino acid signal peptide, was used to replace the Mo-MuLV *env* sequences that had been deleted. An EPO sequence with compatible ends was created by polymerase chain reaction (PCR)-mediated mutagenesis, with 5'-GGCCTC-CCAGTCGTAACCGCCCCCACCGC-3' as the 5' primer and 5'-GGACACTCTGGATCTGTCCC-CTGTCT-3' as the 3' primer, followed by restriction digest of the PCR product with Bst EII and Bam HI, so that it could be inserted by means of the appropriate restriction sites while maintaining the proper reading frame. We also made a second construct containing EPO in the central portion of gp70 (between positions 6257 and 6761 of the *env* gene), directly overlapping a proline-rich hypervariable region. Although a few subclones expressed this construct at the protein level, as shown by protein immunoblot analysis, none expressed it on the surface of the packaging cells, as shown by FACS analysis. This lack of cell-surface expression was presumably due to retention in the endoplasmic reticulum (12).
19. To create packaging cell lines expressing the recombinant envelope,  $1 \times 10^6$   $\psi$ 2 cells, grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 1% penicillin-streptomycin, were cotransfected with 20  $\mu$ g of pEPOenv A5923 and 1  $\mu$ g of pFR400 by means of the calcium phosphate precipitation method (Gibco). The medium was changed 16 to 24 hours later and was subsequently selected with gradually increasing concentrations of MTX (Sigma) from 0.2 to 10  $\mu$ M, at which point subclones were isolated from the surviving colonies and screened by protein immunoblot. Subclones were grown to confluence in 10-cm plates, washed with phosphate-buffered saline (PBS), and lysed in 4% SDS, 10% glycerol, 10%  $\beta$ -mercaptoethanol, and 50 mM tris (pH 6.7) and boiled for 10 min. The samples were subjected to 8% SDS-polyacrylamide gel electrophoresis and electrotransferred to nitrocellulose (Hybond ECL, Amersham). The filters were blocked in TBST buffer [0.02% Tween-20, 150 mM NaCl, and 50 mM tris (pH 7.5)] with 5% dried milk and incubated with a primary EPO mAb (Genzyme), then washed in TBST buffer with 0.5% dried milk and incubated with a secondary horseradish peroxidase-conjugated goat antibody to mouse immunoglobulin G (IgG) (CalTag, San Francisco, CA). After being washed again, the filters were incubated in ECL chemiluminescent immuno-detection reagents (Amersham) and exposed to film. The same specific 70-kD band was also observed when the filters were reblocked and reprobed with a polyclonal goat anti-*env* (Microbiological Associates, Rockville, MD) as the primary antibody, followed by horseradish peroxidase-conjugated swine antibody to goat IgG (CalTag) as the secondary antibody.
20. Polyclonal antiserum to EPO no. 8C295 (Amgen) and fluorescein-conjugated secondary antibodies (CalTag) were used for flow cytometry, carried out with a Becton Dickinson FACScan and FACS IV, operated by P. Dazin.
21. Virus-producing cell lines were created by transient transfection of amphotropic packaging cell line PA317 with pCRIP-SVAc (which contains the gene for neomycin resistance and the gene for  $\beta$ -Gal and is a modification of a retroviral vector plasmid provided by R. Scharfman) by means of the calcium phosphate precipitation method. The medium was changed after 24 hours, and the virus-containing medium was filtered through a 0.2- $\mu$ m filter into medium containing polybrene (8  $\mu$ g/ml) and then used to infect wild-type  $\psi$ 2 as well as  $\psi$ EPOenv 8.1 packaging cells 48 hours after transfection. The infected packaging cells were subsequently replated at low density and selected in G418 (400  $\mu$ g/ml) (Gibco), and individual G418-resistant colonies were isolated. Subcloned colonies were retested for expression of the EPO epitope by protein immunoblot and FACS analysis.
- Ampotrophic, wild-type virus-producing cells were created by a similar strategy that used transient transfection of wild-type  $\psi$ 2 cells with pCRIP-SVAc to generate virus for infection of PA317 packaging cells, followed by G418 selection.
22. NIH 3T3 cells were cotransfected with pXM EPO-R and pFR400 and selected in increasing concentrations of MTX ranging from 0.2 to 10  $\mu$ M. Expression of the EPO receptor was assayed by binding of <sup>125</sup>I-EPO peptide (Amersham); target cells were grown to confluence in 24-well plates and subsequently incubated in DMEM with 2% bovine serum albumin (BSA) and approximately 10<sup>6</sup> cpm of <sup>125</sup>I-EPO, with or without excess cold EPO, for 90 min at 37°C in a humidified incubator, washed with PBS, lysed in 1N NaOH, and counted in a gamma counter. The approximate number of EPO receptors per cell was estimated on the basis of the specific activity of the <sup>125</sup>I-EPO and the approximate number of cells contained within the wells. HEL and K562 cells were similarly assayed for <sup>125</sup>I-EPO binding, except that cells were incubated with <sup>125</sup>I-EPO in suspension and were spun for 5 min at 1000 rpm in a Beckman desktop centrifuge to pellet the cells after each wash.
23. The  $\psi$ 2 wild-type control virus showed titers of  $3.1 \times 10^4$  per milliliter and  $2.8 \times 10^4$  per milliliter on NIH 3T3 wild-type and NIH 3T3 + EPO receptor target cells, respectively. The  $\psi$ 2 wild-type virus titers were not affected by the presence or absence of EPO. The EPO-env virus showed a titer of  $1.8 \times 10^4$  per milliliter on NIH 3T3 wild-type cells and  $1.2 \times 10^5$  per milliliter on NIH 3T3 + EPO receptor cells without EPO competition. With EPO competition, the EPO-env virus titers on NIH 3T3 wild-type cells were not significantly affected; however, the titers on NIH 3T3 + EPO receptor cells were drastically reduced, to  $6.2 \times 10^4$  per milliliter with the addition of 5  $\mu$ g of EPO per milliliter and  $5.5 \times 10^4$  per milliliter with the addition of 50  $\mu$ g of EPO per milliliter. Two variables were then taken into account in interpreting these results and determining the efficiency of infection.
- First, differences in initial plating density and growth rates of the two target cell lines were corrected for by comparison of the number of G418-resistant NIH 3T3 + EPO receptor colonies with the number of G418-resistant NIH 3T3 wild-type colonies after infection by  $\psi$ 2 wild-type virus. Second, differences in the overall titers of the wild-type and EPO-env viruses were corrected for by comparison of the number of G418-resistant NIH 3T3 wild-type colonies after infection with  $\psi$ 2 virus with that of NIH 3T3 wild-type colonies after infection with EPO-env virus. Thus, the relative infection efficiency of EPO-env virus on NIH 3T3 + EPO receptor cells as compared with that of  $\psi$ 2 wild-type virus was derived by the following formula: relative infection efficiency = (number of G418-resistant NIH 3T3 + EPO receptor colonies per number of G418-resistant NIH 3T3 wild-type colonies) after  $\psi$ 2 wild-type virus infection ÷ (number of G418-resistant NIH 3T3 + EPO receptor colonies per number of G418-resistant NIH 3T3 wild-type colonies) after  $\psi$ 2 wild-type virus infection. The relative infection efficiency thus derived, expressed as the mean  $\pm$  SE, was  $6.1 \pm 1.2$  ( $n = 6$ ).
24. We thank J. Chang for help in retrovirus work and T. Reudelhuber, D. Gardner, K. Yamamoto, and J. A. T. Young for stimulating discussions and helpful advice. Plasmids containing Mo-MuLV genes were generously provided by A. Bank and D. Markowitz. A plasmid containing EPO cDNA sequences was generously provided by K. Shannon. Plasmids containing the murine and human EPO receptor cDNAs were generously provided by A. DiAndrea. Polyclonal antiserum to EPO no. 8C295 was generously provided by J. Egrie. Supported in part by NIH grants HL 20985 (to the Northern California Comprehensive Sickle Cell Center) and AM16666 (to Y.W.K., who is an investigator at the Howard Hughes Medical Institute).

28 February 1994; accepted 14 September 1994

## Interaction of the p53-Regulated Protein Gadd45 with Proliferating Cell Nuclear Antigen

Martin L. Smith, I.-Tsuen Chen, Qimin Zhan, Insoo Bae, Chaw-Yuan Chen, Tona M. Gilmer, Michael B. Kastan, Patrick M. O'Connor, Albert J. Fornace Jr.\*

**GADD45** is a ubiquitously expressed mammalian gene that is induced by DNA damage and certain other stresses. Like another p53-regulated gene, *p21<sup>WAF1/CIP1</sup>*, whose product binds to cyclin-dependent kinases (Cdk's) and proliferating cell nuclear antigen (PCNA), **GADD45** has been associated with growth suppression. Gadd45 was found to bind to PCNA, a normal component of Cdk complexes and a protein involved in DNA replication and repair. Gadd45 stimulated DNA excision repair in vitro and inhibited entry of cells into S phase. These results establish **GADD45** as a link between the p53-dependent cell cycle checkpoint and DNA repair.

An important cellular response to DNA damage is the arrest of cell cycle progression at  $G_1$  and  $G_2$  checkpoints, which presumably allows time for DNA repair before

M. L. Smith, I.-T. Chen, Q. Zhan, I. Bae, P. M. O'Connor, A. J. Fornace Jr., Laboratory of Molecular Pharmacology, Developmental Therapeutics Program, National Cancer Institute, Building 37, Room 5C09, Bethesda, MD 20892, USA.

C.-Y. Chen and M. B. Kastan, Johns Hopkins Oncology Center, Baltimore, MD 21287, USA.

T. M. Gilmer, Glaxo Research Institute, Research Triangle Park, NC 27709, USA.

\* To whom correspondence should be addressed.

entry into S and M phase, respectively (1). The p53 tumor suppressor is required for one such  $G_1$  checkpoint in mammalian cells (2, 3). After genotoxic stress, p53 functions as a transcription factor and transactivates effector genes such as **GADD45** and *p21<sup>WAF1/CIP1</sup>*, although both of these genes can be induced by other pathways (3, 4). *p21<sup>WAF1/CIP1</sup>* inhibits the kinase activity of multiple Cdk complexes, which may be one mechanism by which it suppresses cellular growth (5, 6), and it inhibits the ability of PCNA to activate

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? s (virus or viral) (5n) vector??  
    968071  VIRUS  
    536607  VIRAL  
    365876  VECTOR??  
    S1  26801  (VIRUS OR VIRAL) (5N) VECTOR??  
? s receptor (5n)target?  
    1455386  RECEPTOR  
    696478  TARGET?  
    S2  14989  RECEPTOR (5N)TARGET?  
? s s1 and s2  
    26801  S1  
    14989  S2  
    S3  183  S1 AND S2  
? s s3 and py<=1994  
Processing  
    183  S3  
    25468438  PY<=1994  
    S4  10  S3 AND PY<=1994  
? rd  
>>>Duplicate detection is not supported for File 340.  
>>>Records from unsupported files will be retained in the RD set.  
...completed examining records  
    S5      8. RD (unique items)  
? t s5/3,k,ab/1-8
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5/3,K,AB/1 (Item 1 from file: 155)  
DIALOG(R) File 155: MEDLINE(R)

08681956 96050944 PMID: 7584085 10/11/02  
Strategies to achieve targeted gene delivery via the receptor  
-mediated endocytosis pathway..  
Michael S I; Curiel D T  
Department of Biochemistry and Molecular Genetics, University of Alabama  
at Birmingham 35294, USA.  
Gene therapy (ENGLAND) Jul 1994 1 (4) p223-32, ISSN  
0969-7128 Journal Code: 9421525  
Document type: Journal Article; Review; Review, Tutorial  
Languages: ENGLISH  
Main Citation Owner: NLM  
Record type: Completed  
Gene transfer to eukaryotic cells may be accomplished by capitalizing on  
endogenous cellular pathways of macromolecular transport. In this regard,  
molecular conjugate vectors have been developed which deliver DNA via the  
receptor-mediated endocytosis pathway. An attractive feature of this vector  
system is the potential to achieve targeted gene delivery based upon  
flexible incorporation of a targeting ligand. In this review we describe  
steps that have been taken to optimize this vector system. Specific  
strategies include the incorporation of mechanisms to achieve conjugate  
escape from the endosome and the derivation of methods to eliminate sources  
of nonspecificity. These developments have demonstrated the potential to  
construct a vector system in which multiple independent components may  
function in a concerted manner to accomplish targeted high efficiency gene  
delivery. In their present state of development, molecular conjugate  
vectors may have many potential applications for in vitro use.

Strategies to achieve targeted gene delivery via the receptor  
-mediated endocytosis pathway.  
Jul 1994,  
Chemical Name: Genetic Vectors; Receptors, Cell Surface; Receptors,  
Virus; Polylysine; DNA

5/3,K,AB/2 (Item 2 from file: 155)  
DIALOG(R) File 155: MEDLINE(R)

*cannot use this*  
*for 103 bec. of*  
*16/16 mutant*  
*Rej.*

08309086 95064013 PMID: 7973726

Tissue-specific targeting of retroviral vectors through ligand-receptor interactions.

Kasahara N; Dozy A M; Kan Y W

Department of Laboratory Medicine, University of California, San Francisco 94143-0724,

Science (UNITED STATES) Nov 25 1994, 266 (5189) p1373-6,  
ISSN 0036-8075 Journal Code: 0404511

Contract/Grant No.: AM16666; AM; NIADDK; HL 20985; HL; NHLBI

Comment in Science. 1994 Nov 25;266(5189) 1326; Comment in PMID 7973723;  
Comment in Science. 1995 Jul 21;269(5222):417; Comment in PMID 7618110

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The development of retroviral vectors that target specific cell types could have important implications for the design of gene therapy strategies. A chimeric protein containing the polypeptide hormone erythropoietin and part of the env protein of ecotropic Moloney murine leukemia virus was engineered into the virus. This murine virus became several times more infectious for murine cells bearing the erythropoietin receptor, and it also became infectious for human cells bearing the erythropoietin receptor. This type of tissue-specific targeting by means of ligand-receptor interactions may have broad applications to a variety of gene delivery systems.

Tissue-specific targeting of retroviral vectors through ligand-receptor interactions.

Nov 25 1994,

...bearing the erythropoietin receptor, and it also became infectious for human cells bearing the erythropoietin receptor. This type of tissue-specific targeting by means of ligand-receptor interactions may have broad applications to a variety of gene delivery systems.

Descriptors: Erythrocytes--virology--VI; \*Genetic Vectors--genetics--GE; \*Moloney murine leukemia virus--genetics--GE; \*Receptors, Erythropoietin--metabolism--ME

Chemical Name: Chimeric Proteins; Genetic Vectors; Ligands; Receptors, Erythropoietin; Receptors, Virus; Viral Envelope Proteins; Erythropoietin

5/3,K,AB/3 (Item 3 from file: 155)  
DIALOG(R) File 155: MEDLINE(R)

07968095 94107310 PMID: 8280129

Repression of glucocorticoid receptor function by the anti-rheumatic gold compound aurothiomalate.

Makino Y; Tanaka H; Hirano F; Fukawa E; Makino I

Second Department of Internal Medicine, Asahikawa Medical College, Japan.  
Biochemical and biophysical research communications (UNITED STATES) Dec  
30 1993, 197 (3) p1146-53, ISSN 0006-291X Journal Code: 0372516

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We describe functional interaction between the anti-rheumatic water-soluble gold compound aurothiomalate and glucocorticoid receptors. Aurothiomalate contains gold in the Au(I) state, and Au(I) alone, not thiomalate or malate, reduced the ligand-binding activity of the glucocorticoid receptor. Au(I) also interfered with the productive

interaction between the glucocorticoid receptor and the cognate target DNA sequences and repressed the transactivation function of the receptor. Thus, Au(I) variably modulates glucocorticoid receptor-mediated intracellular signals.

Dec 30 1993,

... of the glucocorticoid receptor. Au(I) also interfered with the productive interaction between the glucocorticoid receptor and the cognate target DNA sequences and repressed the transactivation function of the receptor. Thus, Au(I) variably modulates...

...; Chloramphenicol O-Acetyltransferase--metabolism--ME; DNA-Binding Proteins--drug effects--DE; Dexamethasone--metabolism--ME; Genetic Vectors; Hamsters; Kinetics; Mammary Tumor Virus, Mouse; Molecular Sequence Data; Oligodeoxyribonucleotides; Plasmids; Promoter Regions (Genetics); Receptors, Glucocorticoid--antagonists and inhibitors --AI...

5/3,K,AB/4 (Item 4 from file: 155)  
DIALOG(R) File 155: MEDLINE(R)

07690191 93212537 PMID: 8460503

Expression of the La Crosse M segment proteins in a recombinant vaccinia expression system mediates pH-dependent cellular fusion.

Jacoby D R; Cooke C; Prabakaran I; Boland J; Nathanson N; Gonzalez-Scarano F

Department of Neurology, University of Pennsylvania Medical Center, Philadelphia 19104-6146.

Virology (UNITED STATES) Apr 1993, 193 (2) p993-6, ISSN 0042-6822 Journal Code: 0110674

Contract/Grant No.: AI-24888; AI; NIAID; GM 07170; GM; NIGMS; NS-20904; NS; NINDS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

To study the expression of La Crosse virus (LAC) glycoproteins, G1 and G2, we constructed a cDNA copy of the open reading frame (ORF) of the middle RNA segment and expressed it in a recombinant vaccinia virus (VV. ORF). Cells infected with VV. ORF expressed G1 and G2 at the cell surface and formed syncytia with a pH profile similar to that of LAC. These experiments provide a system of studying the biological functions of the LAC glycoproteins, including processing, targeting, fusion, receptor binding, and antigenicity.

Apr 1993,

... experiments provide a system of studying the biological functions of the LAC glycoproteins, including processing, targeting, fusion, receptor binding, and antigenicity.

Chemical Name: Codon; Genetic Vectors; RNA, Viral;  
Viral Proteins

5/3,K,AB/5 (Item 5 from file: 155)  
DIALOG(R) File 155: MEDLINE(R)

07658694 93176831 PMID: 8382533

Interaction between the thyroid hormone receptor and co-factors on the promoter of the gene encoding phospho enol pyruvate carboxykinase.

Schmidt E D; van Beeren M; Glass C K; Wiersinga W M; Lamers W H  
Department of Anatomy and Embryology, AMC, Amsterdam, Netherlands.  
Biochimica et biophysica acta (NETHERLANDS) Feb 20 1993, 1172  
(1-2) p82-8, ISSN 0006-3002 Journal Code: 0217513

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Using transient transfection studies we localized a thyroid hormone-responsive element on the promoter of the rat phospho-enol pyruvate carboxykinase gene between 355 and 174 bp upstream of the transcription start site. DNase 1 footprinting analysis within this region showed that a 28 bp fragment at position -324 to -297 was protected by the thyroid-hormone receptor. This receptor was overexpressed in HeLa cells using a vaccinia virus expression vector. DNA-binding assays with this receptor-enriched nuclear HeLa cell extract revealed that only 20% of the thyroid hormone receptor was able to bind the target sequence with high affinity (4 nM). Titration with nuclear extract from hepatocytes increased the percentage of c-erbA molecules able to bind to this thyroid hormone-responsive element 4-fold without a change in affinity. Our data show that the complex of the thyroid hormone responsive element of the promoter of the phosphoenol pyruvate carboxykinase gene and the thyroid hormone receptor contains only a single receptor molecule suggesting the formation of a heterodimer complex. Accordingly, this thyroid hormone receptor/DNA complex is formed only in the presence of co-factors that are present in limiting amounts in the hepatocyte nucleus.

Feb 20 1993,

... by the thyroid-hormone receptor. This receptor was overexpressed in HeLa cells using a vaccinia virus expression vector. DNA-binding assays with this receptor-enriched nuclear HeLa cell extract revealed that only 20% of the thyroid hormone receptor was able to bind the target sequence with high affinity (4 nM). Titration with nuclear extract from hepatocytes increased the percentage...

5/3,K,AB/6 (Item 6 from file: 155)  
DIALOG(R)File 155: MEDLINE(R)

06559207 90258935 PMID: 2342476

Specific region in hormone binding domain is essential for hormone binding and trans-activation by human androgen receptor.

Govindan M V

Research Centre, Laval University Medical Centre, Quebec, Canada.

Molecular endocrinology (Baltimore, Md.) (UNITED STATES) Mar 1990  
, 4 (3) p417-27, ISSN 0888-8809 Journal Code: 8801431

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Complementary DNA (cDNA) clones encoding human-androgen receptors (hAR) were isolated using synthetic oligonucleotides homologous to the human glucocorticoid, estradiol, progesterone, and aldosterone receptors as probes to screen a human testis lambda gt11 cDNA library. One of the receptor proteins (hARa) produced in vitro bound the [<sup>3</sup>H]dehydrotestosterone ([<sup>3</sup>H]DHT) with high affinity and selectivity similar to the human androgen receptor present in target tissues and cells. A second cDNA clone (hARb) encoding an identical amino terminal and DNA binding domains, but differing by four amino acids at the hormone binding domain, did not bind [<sup>3</sup>H]DHT with high affinity when incubated with protein expressed by in vitro transcription-translation. Cotransfection of hARa in an expression vector with mouse mammary tumor virus (MMTV)-bacterial chloramphenicol acetyltransferase chimeric plasmids, followed a hormone-dependent trans-activation, defining the binding affinity of hARa between 5 x 10(-10) and 1 x 10(-9) M for [<sup>3</sup>H]DHT. A similar cotransfection experiment with hARb indicated a KD of hARb for [<sup>3</sup>H]DHT to be above approximately 10(-8) M. The deduced primary structures of hARa and hARb contain the viral erbA homologous region found in other steroid, thyroid, and vitamin receptors and is identical to the hAR sequences reported by others. The amino acid sequence differs at the

Gly stretch (16 Gly instead of 27, 24 or 23) of the N-terminal domain and in hARb, the sequence reads I.F.F.F.F.L.L (816-822) instead of K.F.F.D.E-L (816-821) in the hARa and other reported hAR sequences. The difference of four amino acids in the steroid binding domain of hARb is associated with altered DHT binding and thus a lack of trans-activation by way of AR responsive elements in MMTV-long terminal repeat. The interaction of hARa and hARb with synthetic responsive elements by gel-retardation assay and their responsiveness in trans-activation by calcium phosphate coprecipitation demonstrates that hARb can inhibit trans-activation by hARa in this system.

Mar 1990,

... the [<sup>3</sup>H]dehydrotestosterone ([<sup>3</sup>H]DHT) with high affinity and selectivity similar to the human androgen receptor present in target tissues and cells. A second cDNA clone (hARb) encoding an identical amino terminal and DNA...

... incubated with protein expressed by in vitro transcription-translation. Cotransfection of hARa in an expression vector with mouse mammary tumor virus (MMTV)-bacterial chloramphenicol acetyltransferase chimeric plasmids, followed a hormone-dependent trans-activation, defining the binding...

5/3, K, AB/7 (Item 1 from file: 34)  
DIALOG(R) File 34: SciSearch(R) Cited Ref Sci  
(c) 2002 Inst for Sci Info. All rts. reserv.

03206544 Genuine Article#: NN472 Number of References: 33  
Title: INCREASED CYTOTOXICITY OF INTERLEUKIN-2 PSEUDOMONAS EXOTOXIN (IL2-PE) CHIMERIC PROTEINS CONTAINING A TARGETING SIGNAL FOR LYSOSOMAL MEMBRANES (Abstract Available)  
Author(s): FISHMAN A; BARKANA Y; STEINBERGER I; LORBERBOUMGALSKI H  
Corporate Source: HEBREW UNIV JERUSALEM, HADASSAH MED SCH, DEPT CELL BIOL/IL-91120 JERUSALEM//ISRAEL//; HEBREW UNIV JERUSALEM, HADASSAH MED SCH, DEPT CELL BIOL/IL-91120 JERUSALEM//ISRAEL//  
Journal: BIOCHEMISTRY, 1994, V33, N20 (MAY 24), P6235-6243  
ISSN: 0006-2960

Language: ENGLISH Document Type: ARTICLE  
Abstract: IL2-PE40 is a chimeric protein composed of human interleukin 2 (IL2) genetically fused to the amino terminus of a truncated form of pseudomonas exotoxin lacking its cell recognition domain (PE40). IL2-PE40 is extremely cytotoxic to IL2 receptor positive cells. This chimeric protein was found to be an effective and selective immunosuppressive agent for IL2 receptor targeted therapy in many models of disorders of the immune response where activated T-cells play a crucial role. In an attempt to produce an improved IL2-PE40 chimeric protein, we constructed new IL2-PE derivatives. This was done by inserting defined DNA sequences within the chimeric gene encoding IL2-PE40. Inserted sequences represent motifs of other proteins known to be targeted and/or sorted to specific compartments inside or outside the cell. One of the proteins, IL2-PE40(LAP+DUP), containing a targeted signal for lysosomal membrane, was 2-3-fold more active than IL2-PE40. The insertion of the LAP sequence also increased the cytotoxicity of another IL2-PE derivative, IL2-PE66(4Glu). Our results suggest that a selective targeting of IL2-PE chimeric proteins to lysosomes may enable the proteins to reach the cytosol more efficiently, thus improving its specific cytotoxicity. The LAP (lysosomal alkaline phosphatase) sequence may be used as a common motif for increasing the cytotoxicity of other chimeric proteins to be used for targeted immunotherapy.

, 1994

...Abstract: This chimeric protein was found to be an effective and

selective immunosuppressive agent for IL2 receptor targeted therapy in many models of disorders of the immune response where activated T-cells play...  
...Research Fronts: PROTEIN-B; CASEIN KINASE-II; CATALYTIC SUBUNITS)  
92-8077 001 (EXPRESSION OF A RECOMBINANT GENE; VIRAL ASSEMBLY  
PROTEIN; VACCINIA VIRUS VECTORS; DNA-BINDING INVITRO;  
XENOPUS OOCYTES; DIFFERENT EXTRACELLULAR DOMAINS)

5/3,K,AB/8 (Item 1 from file: 340)  
DIALOG(R) File 340:CLAIMS(R)/US Patent  
(c) 2002 IFI/CLAIMS(R). All rts. reserv.

Dialog Acc No: 2525462 IFI Acc No: 9421432  
Document Type: C

DNA ENCODING AN INSECT OCTOPAMINE RECEPTOR; GENETIC ENGINEERING; ADENYLATE CYCLASE INHIBITORS

Inventors: Fraser Claire M (US); McCombie William R (US); Venter John C (US)

Assignee: U S of America Health & Human Services

Assignee Code: 06814

Publication (No,Date), Applic (No,Date):

US 5344776 19940906 US 91676174 19910328

Publication Kind: A

Calculated Expiration: 20110906

(Cited in 001 later patents)

Priority Applic(No,Date): US 91676174 19910328

Abstract: The present invention pertains in general to invertebrate octopamine receptor proteins and to polynucleotides encoding such receptors. The present invention also relates to insect for example, Drosophila octopamine receptors that are recombinantly expressed in mammalian cells where the receptor mediates the attenuation of adenylate cyclase activity and exhibits a pharmacological profile that is unique but closely related to mammalian adrenergic receptors. The present invention further relates to drug screening methods for the development of specific human pharmacological drugs and insecticides **targeted** for the octopamine **receptor** system.

Publication (No,Date), Applic (No,Date):  
...19940906

Abstract: ...relates to drug screening methods for the development of specific human pharmacological drugs and insecticides **targeted** for the octopamine **receptor** system.

Non-exemplary Claims: ...DNA molecule according to claim 5 wherein said vector is a plasmid, bacteriophage or eucaryotic **virus** vector.

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